

Effects of Cadmium Ingestion in Rats with Opposite Genetic Predisposition to Hypertension

by Edward V. Ohanian* and Junichi Iwai*

This study was undertaken to explore the effects of chronic low-level cadmium ingestion in Dahl hypertension-resistant (R) and hypertension-sensitive (S) lines of rats. Groups of weanling female R and S rats were given 0 or 1 mg cadmium/l. in drinking water and fed either a low salt (0.4% NaCl) or a high salt (4% NaCl) diet for 28 weeks.

Cadmium produced hypertension associated with gross cardiac hypertrophy and mild to moderate renal vascular changes in S, but not in R, rats on a low salt diet. Cadmium enhanced the rate and degree of development of salt-induced hypertension without exacerbating the hypercholesterolemia or renal vascular lesions normally observed in S rats on a high salt diet. Cadmium lowered circulating cholesterol levels in both lines on a low salt diet. Cadmium had no influence on growth, blood urea nitrogen concentration, plasma renin activity, tumor formation, or survivorship in R and S rats on either salt diet. This study indicates that the genetic composition is a critical determinant of the adverse effects of chronic low-level cadmium ingestion in rats. In addition to the experimental implications, these findings may have relevance to the problem of human "essential" hypertension.

Introduction

Cadmium, an environmental pollutant, has been implicated in the etiology and pathogenesis of "essential" hypertension in man (1). In experimental studies, hypertension associated with other pathophysiological changes was obtained in rats exposed to cadmium either by chronic ingestion (2, 3) or by parenteral injections (4, 5). However, several other clinical and experimental investigations were unable to replicate these findings (6-11). In our laboratory, we have evolved two unique lines of rats, by selective inbreeding, from a common pool of Sprague-Dawley ancestors. They are designated Dahl hypertension-resistant (R) and hypertension-sensitive (S) lines of rats because of their resistance and susceptibility to develop experimental hypertension in response to excess dietary salt (NaCl) intake as well as to other hypertensinogenic stimuli including intra-arterial and intraperitoneal cadmium injections (12-15). Therefore, it seemed worthwhile to determine whether chronic low-level cadmium

feeding, simulating human exposure, induces biochemical and pathophysiological changes in Dahl rats on low and high salt diets.

Materials and Methods

All the rats used in this experiment were from the two Dahl lines described above. Details on animal care, blood pressure measurements, and the rationale for defining "hypertension" have been given in earlier papers (16-18). Only details pertinent to the present study are therefore included here. Eighty weanling (3-week-old) female R and S rats were divided into four groups, as shown in Table 1. Cadmium, as the acetate, was dissolved in the drinking water (tap water containing 0.0005 to 0.0007 mg cadmium/l.). The low salt (0.4% NaCl) and high salt (4% NaCl) diets were especially ordered (Agway, Inc., Country Foods Division, Syracuse, N. Y.), and their cadmium content was approximately 0.08 $\mu\text{g/g}$ wet weight. The low salt diet contained sufficient sodium for normal growth and development. The specified salt diets and drinking water were available *ad libitum*. All animals were housed in stainless steel cages in air-conditioned rooms maintained at 22°C and 50% relative humid-

* Medical Research Center, Brookhaven National Laboratory, Upton, New York 11973.

Table 1. Classification of groups by salt diet and cadmium in drinking water.

| Group | Diet | Cadmium in drinking water, mg/l. | No. of rats in group | |
|-------|-----------|----------------------------------|----------------------|----|
| | | | R | S |
| I | Low salt | 0 | 10 | 10 |
| II | Low salt | 1 | 10 | 10 |
| III | High salt | 0 | 10 | 10 |
| IV | High salt | 1 | 10 | 10 |

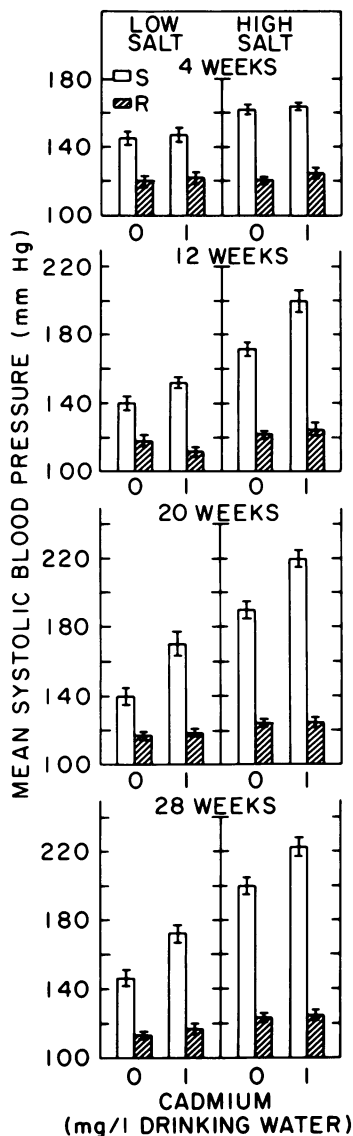


FIGURE 1. Effect of chronic cadmium feeding on blood pressure in R and S rats on low and high salt diets. Each bar represents the mean \pm SEM of 10 animals.

ity with artificial light on for 9 hr and off for 15 hr. Stringent precautions were taken to avoid unnecessary metallic contamination.

At 4, 12, 20, and 28 weeks postweaning, systolic blood pressure of all rats was measured under light ether anesthesia by tail plethysmography (19); body weight was recorded concomitantly with blood pressure; and the volume of water drunk by each group of rats was measured for 24 hr. In addition, at week 28, 1 ml blood was obtained by nicking the tail at the end of the blood pressure measurement, when the rat was still anesthetized, for plasma cholesterol and blood urea nitrogen determination on the AutoAnalyzer II (Technicon Instruments Co., Tarrytown, N.Y.). Also, the urinary bladder was emptied by gentle manual pressure and fresh and uncentrifuged urine was assayed semiquantitatively with reagent strips (Labstix, Ames Co., Elkhart, Ind.) for pH, glucose, and blood. At the end of week 28, 0.5 ml blood was collected from the vena cava of lightly anesthetized rats for renin assay by the technique of Haber et al. (20) [New England Nuclear angiotensin I (125 I) radioimmunoassay kit]. Following the blood collection, the animals were sacrificed and autopsied. Kidneys, liver, heart, and adrenals were excised, trimmed, blotted, and weighed. Furthermore, portions of kidneys and liver were sliced into segments not thicker than 2 mm and placed in 10% neutral formalin; after fixation the sections were stained with hematoxylin and eosin for microscopic examination. Pathological study of tissue sections was made without knowledge of treatment. The remainder of renal and hepatic tissues were stored frozen at -20°C for later cadmium analysis by atomic absorption spectrophotometry (Perkin-Elmer 503) after wet ashing with concentrated nitric acid according to the method of Slavin et al. (21). Statistical analysis was made by Student's *t*-test, chi-square test, or analysis of variance where applicable. The values shown represent the mean \pm standard error of the mean (SEM). A *p* value < 0.05 was considered significant.

Results

Cadmium Ingestion from Drinking Water

In any one group, there was no significant difference in water intake between R and S rats. For the two groups (I and II) fed a low salt diet, the average intake was approximately 15 ml/rat/day which was 1.5 times less than for the corresponding groups III and IV fed a high salt diet. Water intake was used as an index of cadmium ingestion; for example, groups II and IV ingested about 3 and 5 mg of cadmium, respectively, at the end of 28 weeks of cadmium feeding.

Systolic Blood Pressure

The effect of chronic cadmium feeding on systolic blood pressure in R and S rats is illustrated in Figure 1.

Low Salt Diet. Among R and S rats given 1 mg cadmium/l., blood pressure, as compared with that of their respective controls (0 mg cadmium/l.) was not significantly changed after 4 weeks of observations. However, at week 12, cadmium-fed S rats exhibited significantly higher ($p < 0.01$) blood pressure than that of S controls. This cadmium-induced hypertension in S rats persisted through week 28. By contrast, chronic cadmium ingestion had no influence on blood pressure in R rats.

High Salt Diet. S rats in the control group III (0 mg cadmium/l.), as expected (17), developed fulminating hypertension, whereas R rats in groups III and IV, regardless of excess dietary salt and cadmium ingestion, remained normotensive throughout the study. After 12 weeks and thereafter, cadmium-fed (1 mg/l.) S rats manifested elevations of blood pressure which differed significantly ($p < 0.01$) from those of S controls.

Morbidity and Mortality

The animals were alive and in good health until the 26th week of cadmium treatment. Thereafter poor care of the coat and respiratory difficulty were observed to the same extent among groups I to IV and were not aggravated by cadmium. When the experiment was terminated at week 28, two S rats and one R rat in group I were dying, and among the R and S survivors none appeared healthy.

Body and Organ Weights

Body, renal, and hepatic weights of cadmium-fed R and S rats in groups II and IV and their corresponding controls in groups I and III were similar ($p > 0.05$) at autopsy (week 28). The adrenals of groups I and IV S rats (25.2 ± 0.8 mg/100 g body weight; $n = 40$) were identical ($p > 0.05$) and significantly heavier ($p < 0.001$) than those of the counterpart R rats (19.5 ± 0.7 mg/100 g body weight, $n = 40$). Similarly, the hearts of S rats, irrespective of the salt diet and cadmium feeding, were markedly enlarged ($p < 0.01$) when compared with those of R rats (Fig. 2). Significant cadmium-induced gross cardiac hypertrophy ($p < 0.01$) was observed in S rats on a low salt diet.

Plasma Cholesterol and Renin Activity

Cadmium significantly depressed ($p < 0.01$) plasma cholesterol levels in both lines on a low salt

diet after 28 weeks of treatment (Fig. 3). In contrast, chronic excess salt feeding, regardless of cadmium intake, caused significant rise ($p < 0.01$) in plasma cholesterol in S rats as compared with the counterpart R rats.

In any one group, plasma renin activities were significantly elevated ($p < 0.001$) in R rats than they were in S rats. Specifically, the values for R and S rats in groups I to IV were 24.1 ± 4.2 and 10.3 ± 2.6 ; 22.5 ± 5.1 and 12.4 ± 3.1 ; 20.2 ± 3.6 and 5.2 ± 1.1 ; and 19.0 ± 4.3 and 4.7 ± 1.13 ng/ml/hr ($n = 10$), respectively. These observations support our earlier observations in R and S rats on both salt diets (22).

It is noteworthy that none of the rats within groups I to IV exhibited hematuria, glycosuria,

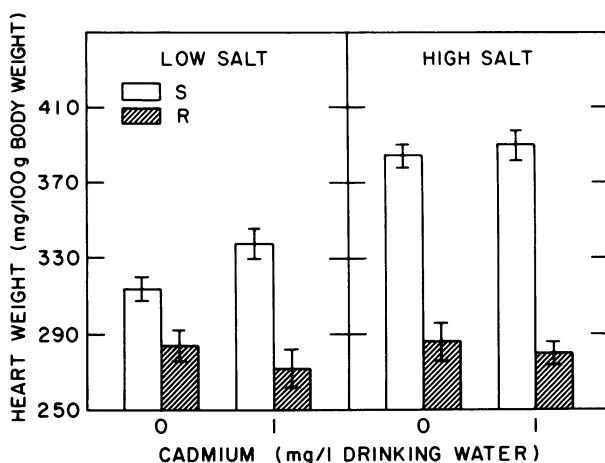


FIGURE 2. Heart weights of R and S rats fed cadmium for 28 weeks. Each bar represents the mean \pm SEM of 10 animals.

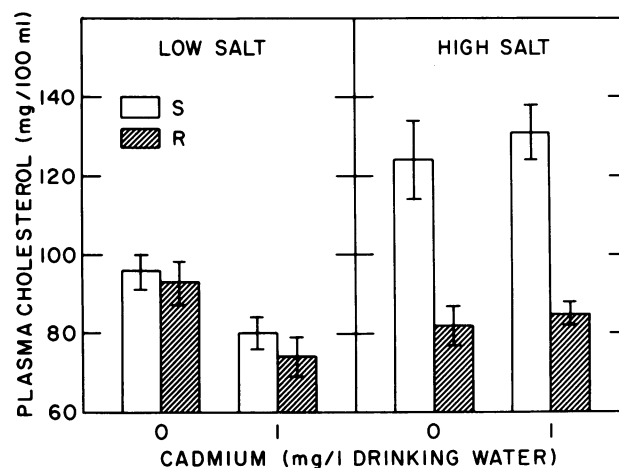


FIGURE 3. Plasma cholesterol levels of R and S rats fed cadmium for 28 weeks. Each bar represents the mean \pm SEM of 10 animals.

azotemia, or altered urinary pH, which was approximately 5.8. Therefore, these data are not discussed further.

Pathological Findings

Tumor Formation. The total incidence of grossly evident tumors in both living and autopsied animals was recorded. Groups I to IV manifested similar incidences of only large mammary tumors. At weeks 12 and 28, the cumulative incidences for these 80 rats were 5 and 10%, respectively.

Periarteritis Nodosa. Of those rats examined macroscopically at autopsy, 30% of groups III and IV S rats, regardless of cadmium ingestion, showed aneurysmal beading of the mesenteric arteries resembling the lesions of periarteritis nodosa.

Renal Vascular and Hepatic Changes. Microscopic changes of glomeruli (thickening of capillary basement membrane; hyaline deposits along the basement membrane; increase in number of epithelial cells; hyalinization and atrophy); tubules (dilatation and casts); and arterioles and arteries (hyperplasia of intima; hypertrophy of media; sclerosis; narrowing of the lumen; necrotizing lesions) were hitherto classed as "renal vascular changes" and graded as follows: mild = +, moderate = ++, and marked = +++. A summary of the renal vascular changes in female R and S rats is shown in Table 2. S rats in group II exhibited mild to moderate (+ - ++), but not marked, changes significantly greater ($p < 0.01$) than those observed in S controls in group I. By contrast, marked renal vascular changes occurred to the same extent in all S rats in groups III and IV. No significant changes were observed in R rats on either salt diet.

Since mild to moderate hepatic changes (cirrhosis, fatty degeneration and vacuolation of hepatic cells) were observed to the same extent in R and S rats in groups I to IV, these data are not tabulated.

Cadmium Concentrations in Kidneys and Liver

The observed kidney and liver cadmium concentrations are presented in Figure 4. Left and right kidneys were analyzed separately. No differences in cadmium levels were noted, hence the results were pooled for further analysis. The presence of small amounts of cadmium in the commercial rat food probably accounts for the detection of the metal in the organs of R and S controls (0 mg cadmium/l.). Cadmium levels in renal and hepatic tissues of S rats fed cadmium and either salt diet were significantly higher ($p < 0.001$) than those of R rats.

Discussion

The results of the current study confirmed, strengthened, and extended the findings of other investigators using rats without opposite genetic propensities for experimental hypertension. In long-term studies, for instance, Schroeder and associates (3, 23-25) found that the feeding of 5 mg cadmium/l. drinking water produced hypertension, renal vascular lesions, hepatic damage, hypocholesterolemia, and cardiac hypertrophy in Long-Evans rats raised in metal-free quarters. Using experimental conditions and models identical to Schroeder's, Perry and co-workers (2) demonstrated that cadmium in drinking water at 1 to 5 mg/l. caused a significant rise on blood pressure without any apparent toxic manifestations, whereas at 10 and 25 mg/l. it had no significant pressor effect. Also, in an earlier study (26), these authors found elevated plasma renin activity in their rats following 1 week and 1 month but not 3 months of cadmium (5 mg/l.) ingestion. Furthermore, Fowler et al. (27) noted that, when Charles River rats were fed 0, 0.2, 2, 20, or 200 mg cadmium/l. drinking water and a normal or low calcium diet, constriction of

Table 2. Renal vascular changes in R and S rats fed cadmium for 28 weeks.

| Group | Diet | Cadmium in drinking water, mg/l. | Rats (n) ^a | Renal vascular changes | | |
|-------|-----------|----------------------------------|-----------------------|------------------------|----------------|-----|
| | | | | 0 | + - ++ | +++ |
| I | Low salt | 0 | R (10) | 10 | 0 | 0 |
| | | | S (10) | 7 | 3 | 0 |
| II | Low salt | 1 | R (10) | 9 | 1 | 0 |
| | | | S (10) | 3 | 7 ^b | 0 |
| III | High salt | 0 | R (10) | 9 | 1 | 0 |
| | | | S (10) | 0 | 3 | 7 |
| IV | High salt | 1 | R (10) | 10 | 0 | 0 |
| | | | S (10) | 0 | 2 | 8 |

^a n = Number of rats examined.

^b = $p < 0.01$ compared with group I, S rats.

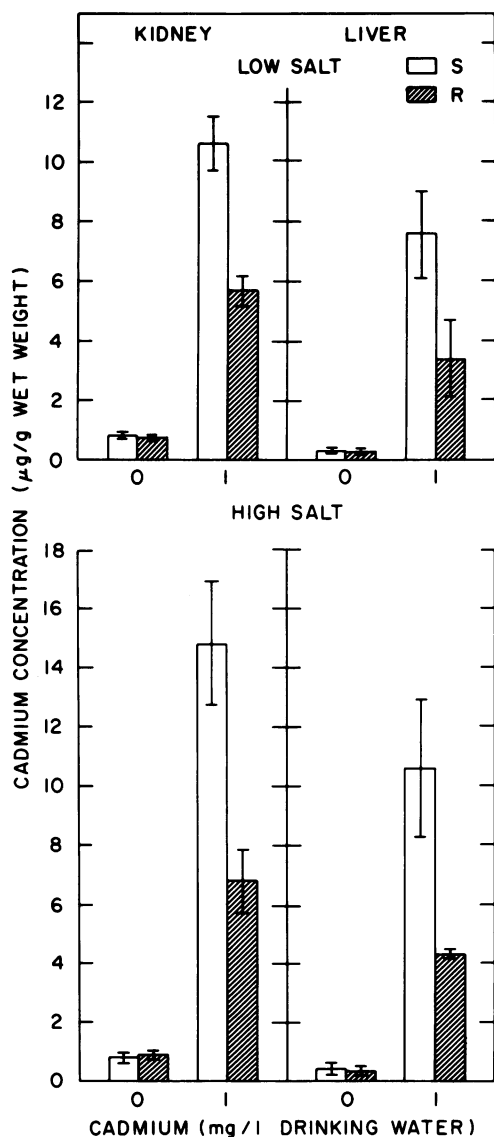


FIGURE 4. Cadmium concentrations in the kidneys and liver of R and S rats fed cadmium for 28 weeks. Each bar represents the mean \pm SEM of 10 animals.

small renal arteries, dilatation of large arteries, and a dose-related scarring of peritubular capillaries were discernible in cadmium-treated rats on both calcium diets after 6 and 12 weeks. Blood pressures were not measured. Conversely, other experimental studies (7-9) were unable to reproduce these adverse effects of cadmium in three different strains of rats, Long-Evans, Sprague-Dawley, and Wistar, given Schroeder's usual dose, 5 mg cadmium/l. in drinking water.

Previously, Dahl and Heine (18) found that the hypertension produced in Sprague-Dawley rats by feeding sea salt (7.3% NaCl) was more pronounced

than that produced by excess dietary salt (8% NaCl) intake alone, although the average amount of NaCl contained in the sea salt feeding was slightly lower. They suspected that other ions might have added to the severity of salt-induced hypertension. However, they did not indicate which ion or ions might be involved. In the present work, the potentiation of the hypertensinogenic effect of high salt (4% NaCl) by cadmium feeding in S rats suggests that the presence of cadmium in sea salt might have been one of the factors that accelerated the rate and degree of development of hypertension in Sprague-Dawley rats. Furthermore, the malignancy of salt-induced hypertension afflicting S rats apparently obscured the incremental effects of cadmium observed in S rats on a low salt diet. It is therefore fair to assume that the addition of 4% NaCl to the regimen of S rats had more devastating effects than chronic low dietary salt (0.4% NaCl) and cadmium ingestion. These experimental findings prove the thesis that hypertension is multifactorial in origin; i.e., there are multiple factors that may operate singly or in combination to induce hypertension in an individual with appropriate genetic predisposition. For example, cadmium together with salt, another environmental contaminant in food, might comprise a particularly vicious combination for inducing hypertension and its complications in predisposed individuals.

It is noteworthy that cadmium levels in the kidneys and liver of hypertensive S rats were approximately twice as high as those of normotensive R rats. Since some published reports showed that metallothionein, a cadmium-binding protein of low molecular weight (6,000-10,000) first identified by Kagi and Vallee (28), is synthesized in mammalian tissues in response to the feeding or injection of cadmium, but not to any other heavy metals studied (29, 30), we are led to speculate that the difference in cadmium concentrations in tissues between the two lines of rats was due to the difference in cadmium-binding properties of metallothionein. No conclusive evidence is yet available to substantiate such a thesis, hence, further speculation at present would not be rewarding.

Our previous parabiosis experiments (31, 32) indicated that, when R and S rats were united in parabiosis, hypertension could be induced in the R rat normally resistant to it. This occurred either when the rats consumed a high salt diet or when they were on a low salt diet but the S rat had one renal artery constricted and the contralateral kidney removed (Goldblatt procedure). Our interpretation of these findings was that S rats produced a humoral hypertensinogenic factor which could be transmitted in parabiosis and produce hypertension in the R

partner. We also surmised that this factor is involved in the pathogenesis of salt and renal hypertension characteristically observed in nonparabiotic S rats. The results of these parabiosis studies combined with those of this study led us to propose that the adverse effects of cadmium in S rats are associated with increased production and/or release of the humoral factor.

In summary, the data cited in this study indicate that the genetic background is a critical determinant of whether pathophysiological changes including hypertension develop following chronic low-level cadmium feeding. Furthermore, if our experimental models have bearing on the etiology of "essential" hypertension in man, this study suggests that a minimal environmental insult such as cadmium exposure will precipitate hypertension in an individual with genetic predilection to hypertension.

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